

-29-

CLAIMS

1. A gene construction comprising:

- a) a first nucleic acid sequence containing the nucleotide sequence encoding a signal peptide;
- b) a second nucleic acid sequence containing the nucleotide sequence encoding a single-domain recombinant antibody; and
- c) a third nucleic acid sequence containing the nucleotide sequence encoding the C-terminal domain of an autotransporter;

wherein the 3' end of said first nucleic acid sequence is linked to the 5' end of said second nucleic acid sequence and the 3' end of said second nucleic acid sequence is linked to the 5' end of said third nucleic acid sequence.

2. Construction according to claim 1, wherein said signal peptide is selected from the signal peptide of PelB, the signal peptide of OmpA, the signal peptide of the M13 bacteriophage protein 3 and the signal peptide of the maltose binding protein (MBP).

3. Construction according to claim 1, wherein said single-domain recombinant antibody is selected from a natural or modified heavy chain variable domain (V_H) of an antibody, a natural or modified light chain variable domain (V_L) of an antibody, a natural or modified recombinant camelid antibody (V_{HH}), a humanized recombinant camelid antibody, a recombinant antibody of a non-camelid animal capable of interacting in the form of a single-domain with its antigen, an IgNAR single-domain antibody of cartilaginous fish, and combinations thereof.

-30-

4. Construction according to claim 1 or 3, wherein said second nucleic acid sequence comprises the nucleotide sequences encoding two or more, equal or different, single-domain recombinant antibodies.

5. Construction according to claim 4, wherein said second nucleic acid sequence comprises the nucleotide sequences encoding three, equal or different, single-domain recombinant antibodies.

6. Construction according to claim 4 or 5, wherein said nucleotide sequences encoding the single-domain recombinant antibodies are separated from each other by nucleic acid sequences encoding a spacer placed between two of said nucleotide sequences encoding the single-domain recombinant antibodies.

7. Construction according to claim 6, wherein said spacer is a peptide sequence having structural flexibility.

8. Construction according to claim 7, wherein said peptide sequence having structural flexibility comprises an amino acid residues multimer or the hinge region of an antibody.

9. Construction according to claim 1, wherein said third nucleic acid sequence comprises the nucleotide sequence encoding the C-terminal domain of an AT of a gram-negative bacteria.

10. Construction according to claim 9, wherein said third nucleic acid sequence comprises the nucleotide sequence encoding the C-terminal β -domain of *Neisseria gonorrhoeae* IgA protease.

-31-

11. Construction according to claim 1, which further comprises a fourth nucleic acid sequence encoding a spacer placed between said second and third nucleic acid sequences, wherein the 5' end of said fourth nucleic acid sequence is linked to the 3' end of said second nucleic acid sequence and the 3' end of said fourth nucleic acid sequence is linked to the 5' end of said third nucleic acid sequence.

12. Construction according to claim 11, wherein said spacer is a peptide sequence having structural flexibility.

13. Construction according to claim 12, wherein said peptide sequence having structural flexibility comprises an amino acid residues multimer or the hinge region of an antibody.

14. Construction according to claim 1, which further comprises a fifth nucleic acid sequence encoding a peptide sequence for detection purposes.

15. Construction according to claim 14, wherein said peptide sequence for detection purposes is a peptide sequence susceptible of being recognized by an antibody.

16. Construction according to claim 15, wherein said peptide sequence susceptible of being recognized by an antibody comprises a poly-hystidine sequence, the E-epitope sequence, the HA epitope sequence, the FLAG epitope sequence or the c-myc epitope sequence.

17. Construction according to claim 14, wherein said fifth nucleic acid sequence is placed between said second and third nucleic acid sequences, wherein the 5' end of said fifth nucleic acid sequence is linked to the 3' end of said second

-32-

nucleic acid sequence and the 3' end of said fifth nucleic acid sequence is linked to the 5' end of said third nucleic acid sequence.

18. An expression vector comprising a gene construction according to anyone of claims 1 to 17 operatively linked to a transcription control sequence.

19. Vector according to claim 18, wherein said transcription control sequence comprises a promoter, a sequence encoding transcriptional regulators, a ribosome binding sequence and/or a transcription terminator sequence.

20. Vector according to claim 19, wherein said transcription control sequence is functional in bacteria and comprises a transcription promoter functional in bacteria, inducible or constitutive.

21. Vector according to claim 20, wherein said promoter is selected from promoter pTetA, promoter pBAD, the promoter of *Escherichia coli lac* operon (pLac), promoter pTac, promoter Pm and the promoter of *E. coli* β-lactamase.

22. Vector according to claim 18, further comprising a marker.

23. An expression vector for single-domain recombinant antibodies on the surface of the bacterial outer membrane (OM), characterized in that the expressed antibody is secreted by a transporter domain of an AT.

24. A bacteria comprising a gene construction according to anyone of claims 1 to 17 or an expression vector according to anyone of claims 18 to 23.

-33-

25. Bacteria according to claim 24, characterized in that is a gram-negative bacteria.

26. Bacteria according to claim 25, selected from a *Escherichia spp.* strain, a *Salmonella spp.* strain and a *Pseudomonas spp.* strain.

27. A hybrid protein obtainable by the expression of the nucleic acid sequence contained in a gene construction according to anyone of claims 1 to 17 or in an expression vector according to anyone of claims 18 to 23.

28. Hybrid protein according to claim 27, comprising a domain (A) which comprises the amino acid sequence of, at least, a single-domain recombinant antibody, and a domain (B) comprising the amino acid sequence of the C-terminal domain of an AT.

29. Hybrid protein according to claim 28, wherein said domain (B) comprises the amino acid sequence of the C-terminal β -domain of *Neisseria gonorrhoeae* IgA protease.

30. Hybrid protein according to claim 28 or 29, further comprising a spacer between said domains (A) and (B) and/or a peptide sequence for detection purposes of said hybrid protein.

31. A method of anchoring and expressing a single-domain recombinant antibody on the surface of the outer membrane (OM) of a bacteria which comprises culturing a bacteria according to anyone of claims 24 to 26, under conditions which allow for the production of said single-domain recombinant antibody, its anchoring and expression on the surface of the OM of said bacteria in the form of a hybrid protein.

-34-

32. A method for the specific adhesion of a bacteria to an antigen which comprises the steps of:

a) transforming a bacteria with a gene construction according to anyone of claims 1 to 17 or with an expression vector according to anyone of claims 18 to 23, said gene construction or expression vector comprising the nucleotide sequence encoding a single-domain recombinant antibody capable of recognizing said antigen;

b) culturing said transformed bacteria under conditions which allow for the production of said single-domain recombinant antibody, its anchoring and expression on the surface of the outer membrane (OM) of said bacteria; and

c) contacting the transformed bacteria and cultured in step b) with said antigen.

33. A method for producing a single-domain recombinant antibody anchored on the surface of the outer membrane (OM) of a bacteria which comprises culturing a bacteria according to anyone of claims 24 to 26, under conditions which allow for the production of said single-domain recombinant antibody, its anchoring and expression on the surface of the OM of said bacteria in the form of a hybrid protein.

34. Method according to anyone of claims 31 to 33 wherein said bacteria is a gram-negative bacteria.